CHAPTER – IV
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Health promoting effects of plants are primarily denoted by the presence of bioactive phytochemicals acting as potent antioxidants having nutritional and pharmacological properties (Harborne and William, 2000; Cai et al., 2004; Han et al., 2007). Among the numerous naturally occurring antioxidants; phenols, flavonoids, tannins and ascorbic acid are most effective one to exert desired physiological effects in human body. In the present study qualitative assessment of phytochemicals of aqueous and alcoholic extracts of *O. sanctum* showed presence of phenols, flavonoids and tannins which were in accordance with the various reported studies (Ramesh and Satakopan, 2010). Phenolics are antioxidants by nature due to their redox properties and metal chelating effects (Rice-Evans et al., 1996; Arlorio et al., 2005). Polyphenols from plant source are excellent free radical scavenger and widely distributed in the herbs of Lamiaceae species (Hakkim et al., 2008). In the present study quantitative estimation of crude polyphenols from aqueous and alcoholic extracts of *O. sanctum* revealed presence of significantly high amount of phytochemicals principally responsible for its protective effect (Table. 3.1).

*In vitro* assessment of antioxidative properties of various plant extracts using chemical models provides biochemical basis for the *in vivo* ethanopharmacological uses of the plants. *O. sanctum* extracts (aqueous and alcoholic) were found to be potent scavenger of superoxide, hydroxyl, nitrous oxide and DPPH radicals. Free radical scavenging effect of *O. sanctum* extracts had also been reported by Hakkim et al. (2007). These scavenging properties are generally due to high reducing capacity of the polyphenols acting as primary antioxidants (Odabasoglu et al, 2004). Metal ions play central role in reactive oxygen species generation as they can change the state from reduced to oxidised causing removal of electron
from various biomolecules (Jomova and Valko, 2011). Both the extracts of *O. sanctum* showed good metal chelating activity as indicated in Fig (3.6). Results of FRAP assay demonstrated that both the extracts (aqueous and alcoholic) possessed an effective capacity for iron binding suggesting its action as reductant. Several studies reported direct correlation between the antioxidant activity and polyphenolic content of the plant extracts which was also seen in present investigation (Juliani and Sinnon, 2002; Kiselova *et al.*, 2007). Strong correlation was found between phenolics of *O. sanctum* extracts and its DPPH radical scavenging effect. Li *et al* (2009) had reported the existence of similar linear correlation between reducing power and total phenolic content (TPC). Table 3.3 showed presence of correlation between total phenolic content and antioxidative effect of aqueous and alcoholic extracts of *O. sanctum*.

Xenobiotic-induced prolonged and unregulated free radical production could be the root cause of numerous degenerative disorders (Murugan *et al.*, 2008) Results showed that butylparaben elevates levels of MDA, a lipid peroxidation product which provides evidence of oxidative injury occurred to the cells and indicates failure of antioxidative defence systems. Accumulation of MDA can cause membrane depolarization, induce enzyme inhibition, modulate transport of protein and ultimately renders membrane integrity (Sen *et al* 2006). Addition of various concentrations of butylparaben reduced activities of antioxidant enzymes such as SOD and catalase significantly in liver homogenates which could be either due to inactivation/denaturation of enzymes or excessively produced reactive oxidative species (ROS). This is in accordance with earlier studies performed in our laboratory where oral administration of paraben (parent acid of butylparaben) - induced oxidative stress in mice liver (Asnani and Verma 2009). Butylparaben treatment to the liver homogenate reduced the levels of non-enzymatic antioxidant and a direct quencher of lipid peroxides—GSH principally because of its excessive utilization by oxidatively rendered hepatocytes.
This could also be due to incorporation of butylparaben moiety into the lipid bilayer of cell that can form covalent bond with sulphydryl groups of membrane molecules such as GSH and prevents it from scavenging the free radicals.

Plants chemicals showing protection against oxidative injury in the biological systems are potential source of biologically active drugs. Aqueous and alcoholic extracts of *O. sanctum* significantly reduced butylparaben-induced lipid peroxidation (Fig. 3.8) due to presence of phytochemicals having strong antioxidative and membrane protecting effect (Nair *et al.*, 1982; Hakkim *et al.*, 2007). Co-treatment of *O. sanctum* extracts significantly restores activities of SOD and catalase may be due to the free radical scavenging activity of the extract polyphenols (Samson *et al.*, 2007). Treatment of aqueous and alcoholic crude phytochemicals of *O. sanctum* stabilizes the levels of total sulphydryl groups in the liver cells majorly responsible for reduction in intrahepatic lipid peroxidation by them (Yanpallewar *et al.*, 2004).

Findings of the another *in vitro* study states that treatment of erythrocytes with H_2O_2 results in 3.47% hemolysis which may be due to reactive oxygen species generation and was significantly higher as compared to control (0.29%). When erythrocytes were treated with 50 μg/ml of butylparaben hemolysis achieved was 1.66% which reached to 7.9% upon H_2O_2 addition in the suspension. Same pattern of hemolysis was followed with butylparaben (100-250 μg/ml) and H_2O_2 combined treatment but with the increased rate. Both the groups (butylparaben and butylparaben+H_2O_2) showed strong correlation (r=0.973) indicating involvement of the same hemolytic mechanism. This accelerated hemolysis could be due to synergistic effect of both the compounds as the maximum concentration of butylparaben need for highest hemolysis was at 250 μg/ml which was reduced to 200 μg/ml in case of (H_2O_2+butylparaben, Fig 3.19). Results of the hemolytic study suggest that butylparaben could generate reactive oxygen species (ROS) involved in lipid peroxidation of membrane.
resulting in leaking of cell content ultimately causing hemolysis. Interaction of \( \text{H}_2\text{O}_2 \) with the iron of heme results in the generation of more potent hydroxyl radical causing hemolysis (Giulivi and Davies, 1994; Nagababu et al., 2003). Same way binding of butylparaben with iron of hemoglobin may interfere with reduction of methemoglobin to oxyhemoglobin resulting in dissociation of hemoglobin subunits and release of iron which itself acts as prooxidant. Hematological studies of butylparaben reported that it can induce tumors of hematopoietic system and also hemolysis in rabbit and human blood which supports our findings (CIR, 1984; Inai et al., 1985).

As shown in Fig 3.20 \( O. \ sanctum \) extract significantly reduces hemolysis in both the groups (butylparaben+extract and butylparaben+\( \text{H}_2\text{O}_2 \)+extract) suggesting its possible role in membrane stabilization. Almost no hemolysis was seen in antidote - treated tubes indicating non-hemolytic activity of \( O. \ sanctum \). This protective mechanism could be due to inhibition of lipid peroxidation and by direct interaction of iron of heme with the reducing components of the plant. Ito et al., (2005) has demonstrated that eugenol, one of the components of \( O. \ sanctum \) can effectively prevent autooxidation of \( \text{Fe}^{2+} \) and keep this transition metal ion at reduced state.

Generally at physiological conditions, erythrocytes have considerable elasticity and fluidity but upon lipid peroxidation the membrane of RBCs gets stiffen and loses its normal morphology and integrity (Ney et al., 1990; Kumar, 2011). Severe cellular deformabilities resulted with butylparaben &/or \( \text{H}_2\text{O}_2 \) treatment could be due to incorporation of butylparaben/\( \text{H}_2\text{O}_2 \) into membrane which can initiate lipid peroxidation making the cell osmotically more fragile. Peroxide formation causes depolarization, alters symmetry and redox potential of the membrane causing swelling and eventually burst of cell resulting in ghost membrane formation (Banerjee et al., 2008). Binding of butylparaben to the outer part of membrane or energy depleting action of it can result in echinocyte formation (Sheetz and
Singer, 1974; Lim et al., 2002). Restoration of normal cellular morphology with *O. sanctum* extract could be due to the polyphenolic component present in it which stabilizes RBC membrane by transferring electron between the hemoglobin-Fe$^{3+}$-butylparaben complex resulting in reduced free radical generation from it (Caragay, 1992).

Accumulation of the lipid peroxidation products in cells/tissues generally gives strong evidence of oxidative injury. Determination of plasma MDA level provides a good measurement of lipid peroxidation in erythrocyte as it could be a result of peroxidation of PUFA present in the membrane. Exposure of blood to different concentrations of butylparaben has resulted in significantly elevated MDA levels (Table 3.3). Correlation analysis had also proven that lipid peroxidation is majorly involved mechanism in butylparaben – induced hemolysis. One of the earlier study performed in our laboratory had shown that p-hydroxybenzoic acid (parent acid of butylparaben) can induce lipid peroxidation in liver and kidney of mice (Asnani and Verma, 2009). Lowering of butylparaben-induced LPO could be the mechanism of protection as strong correlation ($r=0.98$) was also found with *O. sanctum* ameliorated hemolysis and LPO. It was found that with the increasing amount of extract there is increased protection ($r=0.97$) which could be due to increased polyphenolic content of the extract. Resulted reduced lipid peroxidation with *O. sanctum* treatment could be due to large amount of secondary metabolites present in plant extract as they are known to exert antioxidative effect which can terminate series of LPO reactions (Prakash and Gupta, 2009). Geetha *et al.* (2004) had shown that different extracts of *O. sanctum* can effectively reduce LPO in rat blood at very low concentration.

Results of *in vivo* evaluation of butylparaben - toxicity stated that oral administration of low, mid and high dose of butylparaben to mice for 30 days caused significant reduction in body weights of the animals. This reduction could be due to reduced feed intake of the animals (Table 3.5). Results of a sub-chronic study where animals were fed with high dose of
butylparaben showed significant reduction in body weight and body weight gain (Matthews et al., 1956; Daston, 2004). Butylparaben-induced body weight reduction might be due to wasting away of body tissues as Inai et al. (1985) showed atrophy of lymphoidal tissues such as spleen, thymus and lymph nodes with butylparaben (1.25%, 2.5%, 5% and 10%) mixing in animal diet. Multifocal degeneration and significant muscle atrophy was found in liver parenchyma upon butylparaben treatment indicating its tissue destructive effect. Body weight reduction could also be due a result of decrease in muscle mass/protein and carbohydrate turnover of the tissues to meet energy need of the animal.

Treatment of three doses of butylparaben to mice resulted in significant increase absolute and relative weights of mice liver which was principally due to increased levels of hepatic lipids such as cholesterol and triglycerides (Table 3.7). Numerous studies have reported that several xenobiotics induces fatty liver and hypercholesterolemia in experimental animals which gets deposited in the organ ultimately causing increased organ weight of the animal in spite of reduction in body weight (Kataria M and Singh, 1997; Torres-Duran et al., 2006) Subcutaneous administration of butylparaben has been reported to increase uterine mass in ovariectomised and immature rats and mice (Routledge et al., 1998; Soni et al., 2005; Vo et al., 2010). Darbre et al. (2002) had reported that administration of isobutylparaben had increased weights of mouse uterus. Histopathological studies had confirmed that butylparaben could induce hyperlipidemia in mice liver resulting in increased absolute and relative liver weight.

Butylparaben treatment to mice had resulted in reduction in glycogen and protein content in the liver of mice. Reduction in glycogen content could be due altered activities of the enzymes involved in glycogenesis or its increased utilization. Peungvicha et al (1998) that parabens could to increase utilization of glucose resulting in metabolism of hepatic glycogen. It is also possible due to overexpression of cytochrome P450 in butylparaben -
treated liver cells where decrease in serine phosphorylation of glycogen synthase kinase 3alpha results in decreased hepatic glycogen as seen in non-alcoholic fatty liver disease (Kathirvel et al., 2009). Free radicals produced from butylparaben could induce breakdown of glycogen and increase glycogenolysis (Greenvald and May, 1980; Cornoveld et al., 1993).

Thirty days treatment of butylparaben had resulted in elevation of hepatic total lipid and cholesterol content of female mice (Table 3.7). This could be due to inhibitory effect of butylparaben on acetyl-CoA carboxylase, the enzyme required to catalyse the rate-limiting step in hepatic fatty acid synthesis. This inhibitory effect may be either direct, i.e. by an alteration of the enzyme's structure as a result of interaction between toxin and enzyme, or indirect, i.e. through a toxin-induced change in the cellular levels of allosteric effectors of acetyl-CoA carboxylase (Beynen and Geelen, 1982). This aborting effect of paraben on fatty acid esterification in rat hepatocytes might cause accumulation of fatty acid in liver tissue resulting in hepatomegaly. Increase in total lipid and cholesterol contents accompanied by enlargement of liver was confirmed by histological studies. Feeding of xenobiotics might act as stress inducer and stimulate the secretion of catecholeamines and corticosteroid hormones, which induces cholesteropoiesis (Niemann, 1990). Moreover; the low energy status induced by butylparaben worsens mitochondrial damage and promotes the formation of fatty deposits that can progress to cirrhosis. Asnani and Verma (2007) showed that P-hydroxybenzoic acid oral administration increases cholesterol content of mice liver upon thirty days treatment.

Butylparaben administration caused reduction in hepatic DNA, RNA and protein contents the in liver of mice. Butylparaben when activated in liver by mixed function oxidases (cytochrome P 450) can bind with biomolecules (DNA, RNA and protein) by covalent binding. Tayama et al. (2008) showed ability of butylparaben to cause DNA damage which was detectable in Comet assays and could induce chromosome aberrations together with sister-chromatid exchanges. Trace amount of butylparaben was recovered from
breast tumors by Darbre et al. (2004) which gets retained in the lipid portion of the breast tissues and blocks DNA of sweat duct leading to tumor formation. Ishidate et al. (1978) reported the clastogenicity of butylparaben in chromosome aberration tests in Chinese hamster cells. Aberrations studied included chromatid breaks, chromatid gaps, chromosomal exchanges and ring formations. Toxic effects of butylparaben analogs (methyl-, ethyl- and propylparaben) on DNA was also reported by Krauze and Fitak (1971).

P-hydroxybenzoic acid, parent component of butylparaben is known to induce oxidative stress in the biological system (Nishizawa et al., 2006) this could be the reason for rapid depletion in hepatic DNA, RNA and protein content. Free radicals also caused cross-linking and scissoring of DNA strands ultimately affecting replication (Burhans and Weinberger, 2007). Damage caused to RNA by butylparaben generated free radicals could be due to incorporation of oxidized nucleotides during the process of RNA synthesis. Reduced levels of nucleotides affect process of transcription and translation ultimately causing reduction in tissue protein synthesis. Results of present study showed protein reducing effect on butylparaben in mice liver upon 30 days treatment. Under stress condition body constitutes a physiological mechanism with an important role in providing energy from protein catabolism (Smet and Blust, 2001). It is possible that during the metabolism of butylparaben generated free radicals damages mitochondria of liver and prevents them from using sufficient amount of oxygen to produce energy. The protein results suggest that the observed proteolysis is intended to increase the role of protein in energy production during butylparaben-induced stress. The effect of methyl, propyl and butyl esters of p-hydroxybenzoic acid on DNA and RNA synthesis has been tested in toluenized cells of Escherichia coli and Bacillus subtilis. Both RNA and DNA synthesis of these bacteria were inhibited. The inhibitory concentrations were higher than those previously reported for growth inhibition. Protein synthesis in cell-free extracts (S-30 fraction) of B. subtilis was
even more sensitive to parabens than DNA and RNA synthesis, while protein synthesis in *E. coli* was largely unaffected (Ingolf and Trygve, 1983). Asnani and Verma (2006) showed reduction in DNA, RNA and protein content of paraben intoxicated mice liver and kidney.

Oral administration of butylparaben to mice for 30 days had significantly altered the energy status of hepatocytes. Butylparaben treatment resulted in drastically reduced SDH activity - an enzyme bound to inner mitochondrial membrane, which could be due to structural and functional disorganization of the mitochondrial assembly (Fig. 3.10). Nakagawa and Moldeus (1998) showed concentration and time-dependent effect of butylparaben on mitochondrial damage in isolated rat hepatocytes characterised by reduction in ATP levels, loss of total adenine nucleotide pool ultimately inducing cytotoxicity leading cell death. Results of present investigation showed significant reduction in ATPase activity in butylparaben intoxicated animals. This could be due to inhibition of NAD⁺ and FAD-linked respiration and uncoupling of oxidative phosphorylation as reported by butylparaben analog propylparaben (Nakagawa and Moore, 1999) Alteration in mitochondrial potential decreases the rate of cellular ATP synthesis and, thus nucleotide synthesis which may be the root cause of reduction in DNA and RNA content. Energy deficient state of cell characterised by reduced activity of SDH and ATPase could be well correlated with reduction in hepatic protein content (Panet and Atlan, 1979). Mitochondria contents biochemical machinery for oxidation of various biomolecules and produced energy is captured in the form of ATP. Present study reports that butylparaben administration reduces SDH and ATPase activity in mice liver resulting in disturbed metabolism of various biomolecules.

Butylparaben treatment had significantly altered activities of marker enzymes of liver (Table 3.8) in tissue as well as in serum (Table 3.9) indicative of hepatocellular membrane damage and necrosis. Disruption of membrane integrity could be due to butylparaben –
induced oxidative stress causing either leaking out of the content or denaturing of enzyme structure resulting in reduced activities of the enzymes.

Aminotransferases are chemicals liver uses to produce energy from protein muscle mass when low glucose level prevails. ALT activity is an important indice to measure degree of cell mediated damage, while AST is an indicator of mitochondrial damage since it contains 80% of the enzyme. Our study result shows that butylparaben oral treatment significantly increases AST and ALT activity in liver and serum of mice - one more possible reason for protein reduction in butylparaben treated animals. Various hepatocellular researches on human as well as animal models had indicated great correlation between stress and increase in AST, ALT levels (Icen et al., 2005) Disruption of membrane integrity could be due to butylparaben-induced oxidative stress causing either leaking out of the content in blood or denaturing of enzyme structure resulting in reduced activities of the enzymes. γ-GT and ALP are majorly present in linings of bile duct and marked increase is indicative of cholestasis which was found prominently with butylparaben treatment. Serum and hepatic increase was observed in γ-GT and ALP activity indicating possible blockage or injury/inflammation of bile duct. Increase in ACP-lysosomal enzyme was found in butylparaben treated animal’s liver as well as serum samples suggesting significant cellular degeneration and tissue catabolism. Monitoring the level of LDH in liver tissue is an important marker as it is actively involved in glucose metabolism and alteration in its activity is generally indicates xenobiotic/drug-induced hepatic injury (Al-Ghamdi 2003). Butylparaben doses increased activity of liver and serum LDH activity principally to increase energy dependence on glycolysis rather than on aerobic metabolism mostly likely due to mitochondrial damage and depressed TCA cycle enzyme (SDH).

Findings of the present study clearly indicate the involvement of oxidative stress caused by ROS generation in butylparaben – induced hepatotoxicity. Measurement of MDA
levels in the tissue is a great marker of lipid peroxidation, which is among the chief mechanism of cell damage. Findings of the present study clearly indicate the involvement of oxidative stress caused by ROS generation in butylparaben-induced hepatotoxicity. Oral administration of butylparaben for 30 days has resulted in significant elevation in MDA levels which could be due to two possibilities: 1) Either there is increased production of ROS by butylparaben in hepatocytes and/or 2) Suppression of antioxidative system by butylparaben resulting in altered redox potential of cell causing LPO and hence suggesting a considerable hepatocytic oxidative stress. This could be due to lipophilic nature of butylparaben which might allow its easy assimilation in lipid portion of the hepatocytes membrane and initiating chain reactions of LPO (Tavares et al., 2009).

Reduced Glutathione (GSH) and ascorbic acid are important endogenous free radical scavenger and non-enzymatic antioxidants. The levels of GSH and TAA significantly reduced in butylparaben - treated animals (Table 3.11). This reduction might be due to excessively produced free radicals which crosses the scavenging potency of these antioxidants. Nakagawa and Moldeus (1998) had shown reduction in GSH content in butylparaben exposed isolated hepatocytes which support our study. Parabens may engender oxidative stress in the skin following conversion to glutathione conjugates and singlet oxygen (Ishiwatari et al., 2007). Moreover the lipid peroxidation products in liver tissue with GSH results in reduction in free thiol group causing oxidation of this non-enzymatic antioxidant. In the same manner higher amount of peroxide radicals could effectively reduces level of ascorbic acid in butylparaben treated animals.

Liver contains enzymatic antioxidants such as SOD, catalase, GPx, GST which constitutes first line of defence against ROS-induced damage (Kale, 2007) Oral treatment of butylparaben for 30 days significantly reduced the activities of enzymes of antioxidant system (Table 3.11). The depletion enzyme activities might be due to butylparaben-induced
protein oxidation. The interaction of LPO products with enzyme molecules leads to the exclusive modification of histidine residue and generation of protein-protein cross linked derivatives causing reduction in enzyme activity (Wei et al., 2010). Results of the correlation analysis have shown strong negative correlation between LPO and the activities of enzymes. GR contributes to the regeneration of GSH so the suppressed activity of GR could be the reason for the reduction of GSH content. Reduction in activity of antioxidative enzymes renders free radical scavenging potency of the system.

Table 3.12 shows the effect of three different doses of \( O. \ sanctum \) on butylparaben induced body weight reduction. High dose of butylparaben had exerted maximum toxicity and when cotreated with aqueous \( O. \ sanctum \) extract resulted in significant amelioration in body weight of the animals. This protective effect could be due to reduced oxidative stress levels in \( O. \ sanctum \) treated animals resulting in normalization of food intake and metabolism. Sood et al. (2006) have shown protective effect of hydroalcoholic extract of \( O. \ sanctum \) on cardiac changes in rats subjected to chronic restraint stress. The results indicated reduction in animal body weight under chronic stress condition which was successfully ameliorated with cotreatment of 100 mg/kg bw dosage of \( O. \ sanctum \).

Butylparaben treatment had significantly increased the absolute and relative weight of mice liver. Administration of aqueous extracts of \( O. \ sanctum \) in three different doses significantly combated these changes denoting maximum reduction at 300 mg/kg bw dosage (Table 3.13). This effect could be due to lipid lowering effect of the aqueous extract of the plant material. Rai et al (1997) showed that supplementation of \( O. \ sanctum \) leaf powder resulted in significant lowering of total lipid, cholesterol, triglycerides and phospholipids in diabetic rats. This protective effect of \( O. \ sanctum \) was also confirmed by histopathological observations as symptoms of fatty liver and cholesterosis were found to mitigate with plant extract treatment for 30 days.
Table (3.14) shows the effect of aqueous extract of *O. sanctum* on biomolecule contents. High dose of butylparaben had significantly reduced the level of glycogen in mice liver. Oral administration of *O. sanctum* extracts to the butylparaben treated animals resulted in increase in protein and glycogen content of the animals. Grover *et al* (2002) had also reported blood sugar lowering effect of *O. sanctum* which could be due to decrease metabolism of glycogen ultimately increasing the glycogen storage in hepatic tissue. Hypoglycemic effect of *O. sanctum* extract has been reported by numerous scientists in various animal models (Sethi *et al.*, 2004; Hannan *et al.*, 2006) *O. sanctum* leaves caused significant reduction in fasting and post prandial blood glucose level by potentiating the action of exogenous insulin in normal rats (Halim *et al.*, 2001). Restoration of glycogen content could be the reason for decrease in weight loss of the animals by butylparaben treatment.

Butylparaben treatment for 30 days had significantly increased hepatic lipid content and resulted in fatty infiltration of the liver (Table 3.14). Aqueous *O. sanctum* extract when orally given to butylparaben - treated animals in different doses found to reduce this hyperlipidemic effect. This lipid lowering effect of *O. sanctum* was majorly due to phytochemicals present in it. One of the major component of *O. sanctum*- eugenol (essential oil) was found to exert hypoglycaemic and hypolipidemic effect in diabetic animals (Prabhakar and Doble, 2011) This protective effect could be due to presence of some active ingredients and antinutrients such as saponins which helps in improving serum lipid profile and tissue lipid content. Oral administration of aqueous *O. sanctum* extract significantly reduced total cholesterol, LDL, HDL and TAG contents in serum of diabetic animals (Suanarunsawat and Songsak, 2005).

*O. sanctum* extracts are excellent antioxidants and thus prevents the deleterious effects of oxidative stress. Butylparaben treatment for 30 days resulted in significant
reduction in nucleic acid and protein content of the liver and exerted numerous abnormalities of the same (Table 3.14). Cotreatment of aqueous *O. sanctum* extract significantly mitigated these changes majorly due to its antioxidative potency. Antioxidants can interfere with xenobiotic metabolising enzymes, blocks activated mutagens/carcinogens, modulate DNA, RNA repair and even regulate gene expression (Ganasoundari *et al.*, 1997; Siddique *et al.*, 2008) showed radioprotective effect of *O. sanctum* on radiation-induced chromosomal aberrations in mouse bone marrow. Their finding suggests that the decrease in DNA double strand and multiple lesions by *O. sanctum* was proportional to the radiation damage at a given dose suggesting that the protection might be at the primary level of induction of DNA lesion. Pretreatment of rat hepatocytes with *O. sanctum* had significantly reduced levels of DNA adduct and suppressed chemical carcinogenesis (Prashar *et al.*, 1998). Oxidative RNA damage is also a feature in xenobiotic-induced toxicities suggesting that RNA oxidation may actively contribute to the onset or to the development of disease (Nunomura *et al.*, 2006). *O. sanctum* extract was found to increase RNA content of brains exposed to stress (Tabassum *et al.*, 2009). Restored levels of RNA and DNA by *O. sanctum* extract normalises the process of transcription and translation resulting in elevation of protein content. Protein content elevating effect of *O. sanctum* could be due to restored energy status of the tissue resulting in sparing of protein for building muscle blocks. Cotreatment of *O. sanctum* (0.5 g/kg bw) in CCl₄ intoxicated animals resulted in significant increase in hepatic DNA, RNA content and protein synthesis which was reduced in case of toxin treatment only (Pingale, 2010).

Hepatotoxicity of butylparaben was evaluated by estimating the activities of various liver marker enzymes in tissue and serum. Activities of AST, ALT, ALP, ACP, LDH and γ-GT were found to increase with butylparaben treatment in serum as well as liver of mice as indicated in Table (3.14, 3.15). *O. sanctum* extracts are reported to posses antihepatotoxic effects and showed protection against various hepatotoxins (Chattopadhay *et al.*, 1992;
Muglikar et al., 2004). Our findings states administration of all three doses of *O. sanctum* significantly restored back the levels of liver marker enzymes (Table 3.15, 3.16). Activities of AST and ALT-transaminases were found to reduce in extract - treated animals. In the same manner enzymes of bile duct - γ- GT and ALP were found to reduce in both serum as well as liver. Activities of ALP and LDH were also found to restore back with aqueous extract treatment. This effect could be due to membrane protecting potency of the plant extract which prevents leaking out of the content from hepatocytes and prevents necrosis (Lahon and Das, 2011). *O. sanctum* leaf extract reduced activities of AST, ALT, ALP and ACP in serum of paracetamol intoxicated rats (Chattopadhyay et al., 1992). Meera et al. (2009) showed protective effect of *Ocimum* species on CCl₄ and H₂O₂ - induced hepatotoxicity where the activities of AST, ALT and γ- GT were found to reduce with the treatment of 100 mg/kg bw treatment of the extract.

All three doses of butylparaben were found to reduce the activities of SDH and ATPase activity in liver of the animals resulting in altered energy status. *O. sanctum* doses reduced this change in enzyme activity as indicated in Table 3.17. Aqueous extract of *O. sanctum* was found to protect the membrane of the hepatocytes and retards the alteration in protein, carbohydrate and lipid metabolism (Vats et al., 2004) This could be the major factor for elevation of SDH and ATPase activities in liver of *O. sanctum* cotreated mice. Normalised metabolism of protein, carbohydrate and lipid as well as free radical scavenging effect of plant improves integrity and oxidative phosphorylation in mitochondria which was highly disturbed in case of energy deficient state-induced by butylparaben.

Hepatoprotective effect of *O. sanctum* is principally due to its antioxidative potency. Butylparaben treatment in mice elevated levels of lipid peroxidation in liver which was highest with the administration of high dose of butylparaben. *O. sanctum* doses significantly reduced levels of LPO in butylparaben intoxicated animals which could be due to free radical
scavenging effect of *O. sanctum* polyphenols as it was well correlated in our *in-vitro* studies (Table 3.18). Antiperoxidative effect of *O. sanctum* extracts were reported on various stress models by numerous researchers (Kanth and Gupta, 2006; Ghangale *et al.*, 2009) *O. sanctum* pretreatment also stabilizes the levels of tissue total sulfhydral groups during reperfusion and responsible for prevention of reperfusion induced- LPO (Yanpallewar *et al.*, 2004). Content of non-enzymatic antioxidants of the hepatocytes were found to increase with cotreatment of *O. sanctum* extract. The effect was dose-dependent and significant. GSH-major nonprotein thiol in living organism was found to reduce in butylparaben - treated animals which was restored back by free radical scavenging and sulfhydral (thiol) group protecting effects of plant extract. Ascorbic acid content also found to increase due to proton donating effect of *O. sanctum* extract sparing body’s natural antioxidants from getting oxidised. *O. sanctum* extract-induced increase in non-enzymatic antioxidants had been reported by Ramesh and satakopan (2010). Increased level of glutathione could be the reason for reduction in LPO as in presence of GSH lipid peroxides are converted to less toxic alcohol derivatives rather than MDA (Manevich *et al.*, 2002). Activities of enzymatic antioxidants (SOD, CAT, GPx, GR and GST) were found to reduce with butylparaben treatment for 30 days. These enzymes are known to scavenge free radicals such as superoxide, hydroxyl, hydrogen peroxide thus prevents damage caused by oxidative stress to the tissue (Shukla *et al.*, 2004). The present study shows that aqueous extract of *O. sanctum* significantly increases activities of enzymatic antioxidants in butylparaben intoxicated animals. Kusumaran *et al.* (1998) showed the effect of *O. sanctum* extract on chemical carcinogens where *O. sanctum* leaves effectively increased GST activity and protected rats from deleterious effects of carcinogenesis. The levels of thiol group plays vital role in maintaining structural and functional integrity of membranous and enzymatic proteins (Tappel, 1973). This thiol spearing effect of *O. sanctum* extract could be the due to proton donating effect of extract resulting in elevation of reduced glutathione and
ascorbic acid levels ultimately maintaining enzyme protein structure and active site configuration. (Sethi et al., 2007). Aqueous extract of *O. sanctum* is an excellent scavenger of superoxide, hydroxyl, nitrous oxide and DPPH radical as indicated in our *in-vitro* studies. This could be the reason for *O. sanctum*-induced increase in enzymatic antioxidants. Antioxidative effect of *O. sanctum* extract was also responsible for its antiulcer and wound-healing property (Dharmani et al., 2004; Shetty et al., 2006). Increased activities of SOD, and catalase and GSH content along with simultaneous reduction in tissue LPO is principally responsible for protective effect denoted by *O. sanctum* extract against noise-stress and chronic restraint stress. *O. sanctum* mixed in rat diet was also found to increase activities of enzymatic antioxidants under diabetic conditions (Gupta et al., 2006)

Medicinal values of the plants depend on the bioactive constituents exerting desirable physiological action in humans. Antioxidative and hepatoprotective effect of *O. sanctum* extracts are principally denoted by the phytochemicals acting as reductants and free radical scavenger. Though traditionally *O sanctum* - whole herb in its natural form is being used, as synergistic effect of various phytochemicals multiplies the magnitude of the protective effect several times contrary to the isolated compound treatment. However, in depth understanding of mechanisms involved in toxicity as well as preventive effect can be well interpreted in case of isolated pure forms of the compounds. As *O. sanctum* extracts were found to successfully combat butylparaben toxaemia flavonoid from the extract was quantified by HPLC and selected to study the its interaction with butylparaben chemically. Results showed (30%) decrease in absorbance of both the compounds within period of 90 mins indicating presence of some chemical reactions between them which could be the reason for the protective effect of the extract.